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# The Swarming Motility of *Pseudomonas aeruginosa* Is Blocked by Cranberry Proanthocyanidins and Other Tannin-Containing Materials[▽]

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**Bacterial motility plays a key role in the colonization of surfaces by bacteria and the subsequent formation of resistant communities of bacteria called biofilms. Derivatives of cranberry fruit, predominantly condensed tannins called proanthocyanidins (PACs) have been reported to interfere with bacterial adhesion, but the effects of PACs and other tannins on bacterial motilities remain largely unknown. In this study, we investigated whether cranberry PAC (CPAC) and the hydrolyzable tannin in pomegranate (PG; punicalagin) affected the levels of motilities exhibited by the bacterium *Pseudomonas aeruginosa*. This bacterium utilizes flagellum-mediated swimming motility to approach a surface, attaches, and then further spreads via the surface-associated motilities designated swarming and twitching, mediated by multiple flagella and type IV pili, respectively. Under the conditions tested, both CPAC and PG completely blocked swarming motility but did not block swimming or twitching motilities. Other cranberry-containing materials and extracts of green tea (also rich in tannins) were also able to block or impair swarming motility. Moreover, swarming bacteria were repelled by filter paper discs impregnated with many tannin-containing materials. Growth experiments demonstrated that the majority of these compounds did not impair bacterial growth. When CPAC- or PG-containing medium was supplemented with surfactant (rhamnolipid), swarming motility was partially restored, suggesting that the effective tannins are in part acting by a rhamnolipid-related mechanism. Further support for this theory was provided by demonstrating that the agar surrounding tannin-induced nonswarming bacteria was considerably less hydrophilic than the agar area surrounding swarming bacteria. This is the first study to show that natural compounds containing tannins are able to block *P. aeruginosa* swarming motility and that swarming bacteria are repelled by such compounds.**

Bacterial motility plays a pivotal role in microbial surface colonization and the spreading of bacteria across the surface. These motilities contribute to the formation of structured surface-associated communities of bacteria called biofilms (6, 27). Such communities are problematic in a number of environmental and clinical settings due to their enhanced resistance to antimicrobial agents (6, 24, 31). The identification of compounds that impede bacterial motility offers the potential application of such compounds for the limitation of bacterial surface colonization.

Derivatives of cranberry fruit have been identified as compounds that interfere with bacterial adherence to different surfaces, although the effects on bacterial motility are unknown (1, 9, 13, 17, 23, 34, 37). The antiadherence property of cranberry extracts has been attributed largely to specific compounds within the cranberry called proanthocyanidins (PACs) (17). PACs are condensed tannins (a subclass of polyphenol compounds) consisting of catechin and epicatechin monomers that differ from the hydrolyzable tannin structure of ellagitannins that predominate in other fruits, such as pomegranate (29). The monomers of PACs can be linked by one or two intermolecular bonds denoted B-type or A-type linkages, respectively (17). Although, the compositions of tannins in plants can differ substantially (33), cranberry fruit contains predominantly PACs with A-type linkages, while other natural products (such as green tea and grape) have a larger proportion of PACs with B-type linkages (29). The significance of the linkage type has been emphasized by Howell and colleagues who reported that only PACs containing A-type linkages, and not B-type linkages, exhibited antiadherence properties against *Escherichia coli* (18). Evidently, the antiadherence properties of PACs are extremely interesting, but it remains to be determined what effects the cranberry PACs have on levels of bacterial motility.

Bacteria are able to undergo different types of motility that vary depending on the bacterium. Many planktonic organisms are able to initially colonize a surface by utilizing a flagellum to swim toward the surface and attach via bacterial adhesins, such as type IV pili and flagella (6, 26, 27, 28, 32). Once the bacteria are attached, combinations of specific surface-associated motilities, replication (clonal growth), and/or recruitment of additional planktonic bacteria lead to the formation of aggregates of bacteria called microcolonies, that can later lead to mature biofilm development (10, 25, 35). In the case of the Gram-negative bacterium *Pseudomonas aeruginosa*, it can undergo the flagellum-mediated swimming motility and the surface-associated swarming and twitching motilities, which are predominantly mediated by hyperflagellation and type-IV pili, respectively (2, 20).

In this study, *P. aeruginosa* was used as a model organism to investigate the effects that cranberry PACs (CPACs) and pomegranate (PG) ellagitannins have on the different motility types exerted by this bacterium (swimming, swarming, and twitching). After discovering that these compounds completely

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blocked swarming motility, other tannin-containing compounds were subsequently examined for their effects on swarming motility.

## MATERIALS AND METHODS

**Media.** All media were prepared using deionized water (DI; Milli-Q). Bacteria were grown in Luria-Bertani (LB) broth (10 g/liter tryptone, 5 g/liter yeast extract, and 5 g/liter NaCl) and on LB agar (supplemented with 1% [wt/vol] agar) prior to inoculation into the motility assays. Swarm, swim, and twitch media were prepared to assess the corresponding type of motility. Swarm medium consisted of 8 g/liter nutrient broth (Oxoid, United Kingdom) and 0.5% (wt/vol) agar (Fisher Scientific) supplemented with D-glucose (5 g/liter, filter sterilized and added separately). Swim and twitch media consisted of LB broth supplemented with 0.3% and 1.0% (wt/vol) agar, respectively. Tannin-containing compounds or sterile DI was added to the motility media to achieve the concentration that is indicated for each experiment. The surfactant rhamnolipid (Jeneil Biosurfactant Company) was diluted 1:100 into DI to make a 1% (vol/vol) stock solution and filter sterilized. This was later further diluted (1:100) into swarm medium, rendering a final concentration of 0.01% (vol/vol; 100 μl/liter). Swim and twitch plates were dried overnight before use, while swarm plates were dried 1 h before bacterial inoculation. This method of swarm plate preparation was reported to give the most consistent results (36), although we observed the same results on swarm plates that had been dried overnight or for 1 h (data not shown). The capacities of *P. aeruginosa* to grow in the presence of tannins were examined on swarm plates supplemented with both 1.5% and 0.5% (wt/vol) of agar and the indicated tannin solutions. For determination of the planktonic growth rate in broth culture, swarm broth was prepared as for swarm agar, but without the agar.

**Bacterial strains and culture conditions.** Experiments were undertaken with *P. aeruginosa* laboratory strain PAO1, originally isolated from a burn wound (16). Our particular isolate was kindly provided by M. Elimelech from Yale University. Pure stock cultures were maintained at −80°C in 30% (vol/vol) frozen glycerol solutions in LB broth. Frozen cultures were streaked onto LB agar (37°C, 24 h), and a colony of bacteria was inoculated into LB broth (15 ml) to prepare the bacterial inocula for the experiments (37°C, 5 h at 200 rpm).

**Preparation of tannin-containing materials.** The compounds and materials used in this study are as follows. Original CPAC, PG, turmeric (Turm), and cinnamon (Cinn) compounds were kept at room temperature, under no oxygen and protected from light. CPAC was obtained from Amy Howell at Rutgers University. PG, Turm, and Cinn were obtained from Navindra Seeram at the University of Rhode Island. Stock solutions of these compounds were prepared at a concentration of 1.5 mg/ml in DI. Cranberry powder (CP; Atoka Cranberries, Canada) was kept at room temperature and solubilized to achieve a stock concentration of 100 mg/ml in DI. Cranberry tea (CT; Lalco, Canada) and Twinings green tea (TGT; Twinings, England) extracts were created by placing one tea bag in 20 ml of DI (37°C, 30 min) and collecting the dissolved component. Natural cranberry juice (CJ; Mountain River, Canada), diet cranberry juice cocktail (CJC; beverage; Ocean Spray), and Arizona green tea beverages (AGT; Arizona Green Tea, Canada) were also tested. CJC contained sweeteners sucralose and acesulfame potassium, while AGT contained ginseng and honey supplements. The pH of all stock solutions was adjusted to 6.8 to 7.2 with NaOH when required, and the solutions were subsequently filter sterilized with a 0.45-μm filter (Millipore). All solutions were stored at 4°C and protected from light, as PAC compounds are light sensitive (29). Stock solutions of tannin materials were subsequently diluted into the motility media to give the concentration that is indicated for each experiment. Control media were also diluted accordingly with DI. The concentrations reported correspond to the final concentration of tannin material within the motility medium.

**Motility assays.** Motility assays were undertaken in petri dishes (polystyrene, diameter of 82 mm). Swim and swarm plates were inoculated with 5 μl of bacterial broth culture representing approximately $10^8$ CFU/ml. For swim plates, the inoculum was placed directly into the center of the agar so that the motility within the semisolid agar could be evaluated (8). For swarm plates, the inoculum was placed on the agar surface (center) enabling visualization of motility across the agar surface (36). The diameters of the swarming and swimming motility zones were measured after incubation (37°C) for the indicated times. For twitching motility, a colony of bacteria was inoculated deep into the agar with a sterile toothpick so that the toothpick touched the agar-dish interface, and the motility at this interface was subsequently measured (2). To visualize the twitching motility diameter, the agar was carefully removed with tweezers and the plates were stained with 0.1% (wt/vol) crystal violet (1 min) before rinsing.

**Bacterial growth assays.** Swarm plates (with both 1.5% and 0.5% [vol/vol] agar) were supplemented with tannin-containing materials at the indicated concentrations and used to determine the effects of tannins on bacterial growth capacities under conditions relevant to the swarm assays. To do this, bacterial broth culture (identical to that used in the swarm assays) was serial diluted (1:10, up to $10^8$) in sterile phosphate-buffered saline (PBS), and two aliquots (10 μl) of each dilution were spot inoculated onto duplicate plates to verify the highest dilution of bacteria that was able to grow during incubation (16 h, 37°C).

Growth assays were also performed with broth culture, for which aliquots (200 μl) of tannin materials were inoculated into quadruplicate wells of a 96-well microtiter plate (polystyrene; Falcon). Subsequently, an aliquot (5 μl) of a bacterial broth culture (LB, 37°C, 5 h) was inoculated into each well. Covered plates were incubated at 37°C, with shaking at 90 rpm in a microtiter plate reader (Tecan Infinite M200 Pro; Switzerland), with measurements of the optical density at 600 nm ($OD_{600}$) recorded every 20 min for 18 h.

**Filter discs impregnated with tannin-containing materials.** Filter paper discs were impregnated with select natural product solutions to determine whether they would repel swarming bacteria and impair bacterial growth. To do this, filter paper (filter paper 40, ashless; Whatman) was cut to a diameter of 7 mm using a standard hole puncher. Discs were soaked in the indicated solutions, then dried at room temperature (3 h, protected from light), and aseptically placed onto the swarm plates prior to bacterial inoculation. After incubation, it was recorded whether bacteria migrated to or around the filter discs. The exact concentration of the tannin material in the area surrounding the filter disc is unknown but would be expected to be less than the concentration of the solution that the discs were soaked in.

The ability of the discs to impair bacterial growth was evaluated using a disc diffusion assay. Swarm agar (with both 0.5% and 1.5% [wt/vol] agar) was overlaid with an aliquot (2 ml) of bacterial broth culture (prepared as described above). Plates were gently rotated by hand, and excess broth was removed. Filter paper discs were subsequently placed on the plates, and after incubation (16 h), it was observed whether the filter paper discs had resulted in any bacterial growth inhibition zone.

**CA of swarm agar with different tannin-containing materials.** The hydrophilic nature of the swarm agar containing different tannin materials was determined by measuring the contact angle (CA) of water (5 μl water droplets) (pendant-drop technique, OCA 20; Future Digital Scientific) on swarm plates containing different tannin materials. Measurements were made on plates prior to bacterial inoculation and after the swarm assay. Measurements were undertaken on the uncolonized area of agar surrounding the swarm front (if swarming motility occurred) or the area surrounding the central region of bacterial growth (if swarming motility did not occur). Measurements were taken at room temperature.

**Statistical analysis.** When indicated, Student's $t$ test ($P < 0.05$) was used to determine whether the presence of tannin-containing materials resulted in any significant differences compared to when these materials were not present.

## RESULTS

**CPAC and PG blocked swarming motility but did not block swimming or twitching motilities.** Initial experiments investigated the effects that cranberry PAC (CPAC) and pomegranate (PG) extract (100 μg/ml) had on the levels of *P. aeruginosa* strain PAO1 swimming motility and surface-associated swarming and twitching motilities. Under control conditions (in the absence of CPAC or PG), this bacterium was able to undertake all three types of motility (Fig. 1). Interestingly, CPAC and PG both completely blocked swarming motility, even when plates were incubated up to 48 h (Fig. 1A). In contrast, the compounds did not block swimming (Fig. 1B) or twitching (Fig. 1C) motilities. However, at all measured time points, we observed significantly lower levels of swimming motility in the presence of these compounds compared to when no compounds were present ($P < 0.05$). Furthermore, CPAC, but not PG, resulted in significantly reduced twitching motilities at the 72-h time point ($P < 0.05$). However, there were still substantial levels of swimming and twitching motilities in the presence of CPAC or PG (at least 60% of controls), and so the complete

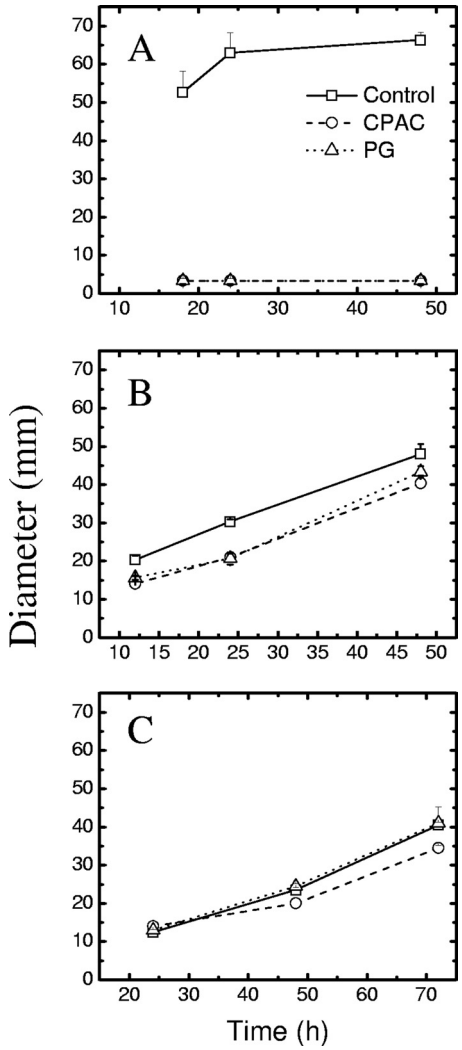


FIG. 1. Effect of CPAC and PG (100 μg/ml) on strain PAO1 swarming (A), swimming (B), and twitching (C) motilities. Values shown represent the mean diameter of corresponding motility zones + standard deviation (SD) of one representative experiment, with triplicate plates per experiment.

blocking of swarming became the key focus of the rest of this study.

Figure 2 shows images of the swarming motility observed in the absence and in the presence of CPAC and PG. Under control conditions, *P. aeruginosa* formed tendrils migrating outwards from the point of bacterial inoculation, with continued branching as the bacteria moved farther from the center (Fig. 2A). This pattern is in agreement with other studies investigating *P. aeruginosa* swarming motility (22, 36). In the presence of CPAC or PG, the bacteria were able to grow and form a colony in the center but exhibited no tendril formation or other features indicative of swarming motility (Fig. 2B and C).

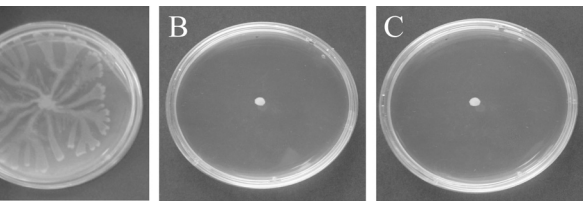


FIG. 2. Representative images of *P. aeruginosa* swarming motility under control conditions (A) and in the presence of CPAC (B) and PG (C) (both 100 μg/ml).

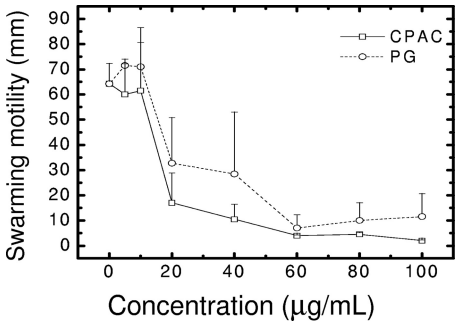


FIG. 3. Effect of CPAC and PG (concentration gradient) on *P. aeruginosa* swarming motility. Values shown represent the mean + SD of 2 experiments, with duplicate plates per experiment.

A range of concentrations of CPAC and PG were tested (5, 10, 20, 40, 60, 80, and 100 μg/ml) to determine the minimum concentrations required to block swarming motility (Fig. 3). As expected, the level of swarming motility decreased as the concentration of each compound increased. CPAC was more consistent in blocking swarming motility than PG (note the error bars in Fig. 3 for PG). For both compounds, a concentration of 60 μg/ml or more virtually blocked swarming motility. Concentrations as low as 20 and 40 μg/ml were also able to significantly impair swarming motility ($P < 0.05$), although there was slight evidence of tendril formation. Lower concentrations (5 and 10 μg/ml) exhibited no effect on swarming levels.

**Some alternative forms of cranberry were able to block swarming motility.** CPAC is a specific fraction of cranberry isolated by complex extraction procedures rendering the product difficult and expensive to obtain for experimental research (18). We tested alternative forms of more readily available cranberry products, namely, crude cranberry powder (CP), cranberry juice (CJ), diet cranberry juice cocktail (CJC), and cranberry tea (CT), to determine whether they could also block swarming motility.

As seen with the CPAC above, CP was very effective at blocking swarming motility (Fig. 4). CJC and CT were also able to impair the level of swarming motility in a dose-dependent manner, although there was some variability in the impairment seen at the lower concentrations of CJC and CT (note the error bars in Fig. 4). The impairment by CJC and CT was not as strong as what was seen with CP and CPAC, possibly because the concentration of PACs could be lower in the former. At certain concentrations, the CJ and CT resulted in no colony growth on the plates (Fig. 4, ≥2.5% CJ and 10% [vol/vol] CT). At lower concentrations, the CJ did not impair or block swarming motility, but a concentration of 5% (vol/vol) for CT resulted in significant impairment ($P < 0.05$). Overall, this demonstrated that different sources of cranberry, but not all, were able to block swarming motility.

We were interested in determining whether another widely

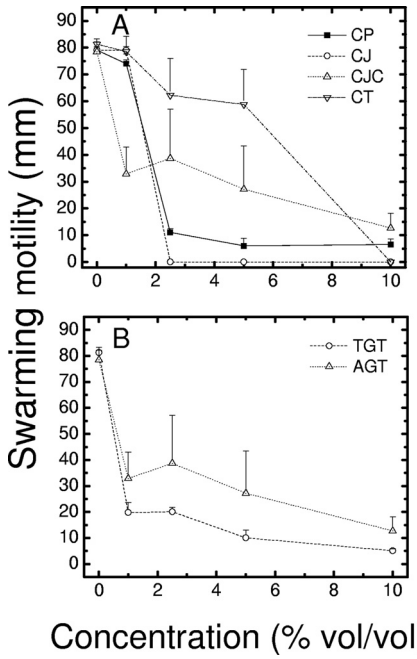


FIG. 4. Effect of a concentration gradient of cranberry products (A) and green tea extracts (B) on *P. aeruginosa* swarming motility. A value of 0 mm denotes that there was no colony growth (as seen for CJ and CT). Values shown represent the mean + SD of two experiments with duplicate plates. Stock solution of CP was 100 mg/ml, so the concentrations in Fig. 4A equate to 1, 2.5, 5, and 10 mg/ml, respectively. All other concentrations equate to dilutions of the relevant stock solution.

consumed tannin material, green tea, would also block swarming motility. Although the composition within green tea varies, it contains a large proportion of catechin-based tannins, made up of B-type PACs (11, 15, 29). Sources of green tea used were TGT (from a teabag) and AGT (iced-tea beverage). Both were able to impair and block swarming motility, albeit the TGT was more effective than the AGT (Fig. 4B). Differences may reflect different concentrations of tannin constituents within the teas, but the results demonstrated that green tea extracts were also able to impair swarming motility.

**Turmeric and cinnamon PACs did not block swarming motility.** Unlike CPAC and PG, cinnamon and turmeric compounds did not block swarming motility at a concentration of 100 μg/ml (data not shown). Cinnamon contains A- and B-type PACs in addition to propelargonidin, which is a subclass of PACs consisting of slightly different subunits termed afzelechin and epiafzelechin (12). Turmeric contains the active ingredient curcumin, which is a tannin-like compound with a composition different than that of PAC (29). This suggests that not all tannin-containing materials are able to block *P. aeruginosa* swarming motility.

**Effect of swarming-blocking materials on bacterial growth rates.** This study revealed that *P. aeruginosa* swarming motility was blocked on plates supplemented with a range of tannin-containing compounds, including CPAC, PG, CP, CJC, TGT, and AGT. As bacterial cells are required to be in contact with one another for swarming motility to occur (5), it was important to ensure that these tannins were not preventing bacterial growth and hence obstructing swarming motility. To investigate this, we examined whether serial dilutions of bacterial broth cultures were able to grow on the swarm plates supplemented with different tannin materials (Table 1). In comparison to control conditions, there was no evidence of growth impairment in the presence of CPAC, PG, CP, CJC, or AGT, as equivalent dilutions of bacteria were able to grow on tannin and control plates. Although the data in Table 1 correspond with an agar level of 1.5% (wt/vol), similar results were seen with plates with an agar concentration of 0.5% (wt/vol) (equivalent to that used in the swarm assays). The challenge in using the lower agar concentration was that in the absence of tannins there was extensive swarming of bacteria on the surface, rendering it difficult to quantify the results (data not shown). In the presence of CJ and CT, the bacteria were not able to grow at all on swarm plates (Table 1), which is in agreement with the findings shown in Fig. 4, for which no bacterial growth was evident at these concentrations of CJ and CT. Bacteria exhibited a reduced growth capacity in the presence of TGT, but the undiluted broth culture (equivalent to that used in the swarm assay) was still able to grow (Table 1). Similar results were also observed when growth rates were determined in the bacterial broth culture assay. CPAC, PG, CP, CJC, or AGT did not impair growth, yet the growth rate was impaired in the presence of TGT (data not shown).

TABLE 1. Bacterial growth capacities on swarm plates supplemented with various tannin-containing materials

| Compound | Concn | No. of CFU/ml[a] |
|---|---|---|
| Control | | $\sim 1.0 \times 10^8$ |
| CPAC | 0.1 mg/ml | $\sim 1.0 \times 10^8$ |
| PG | 0.1 mg/ml | $\sim 1.0 \times 10^8$ |
| CP | 10 mg/ml | $\sim 1.0 \times 10^8$ |
| CJ | 10%, vol/vol | NG |
| CJC | 10%, vol/vol | $\sim 1.0 \times 10^8$ |
| CT | 10%, vol/vol | NG |
| TGT | 10%, vol/vol | $\sim 1.0 \times 10^4$ |
| AGT | 10%, vol/vol | $\sim 1.0 \times 10^8$ |

[a] NG denotes no growth. Values shown equate to the growth level of *P. aeruginosa* per original milliliter of broth culture. Values are representative of duplicate experiments, with duplicate plates and dilutions conducted for each experiment.

These data demonstrate that the majority of tannin-containing materials are not blocking swarming motility by preventing bacterial growth. Furthermore, as will be discussed below, filter paper discs impregnated with different tannin-containing dilutions did not interfere with bacterial growth.

**Tannin-impregnated paper discs repelled swarming bacteria.** We evaluated whether bacteria that were already undertaking swarming motility would be repelled by a point source of the tannin materials. To do this, filter paper discs were soaked in solutions of DI or tannin-containing materials (concentrations equivalent to those described in Table 1), dried, and then placed onto swarm agar prior to inoculation with *P. aeruginosa* (Fig. 5). During incubation, the bacteria would undertake the characteristic swarming motility right up to discs that had been impregnated with DI. In contrast, when the bacteria came into close proximity with discs impregnated with CPAC, PG, CP, CJ, or TGT, they would alter their direction and migrate around the discs (Fig. 5). The swarming bacteria

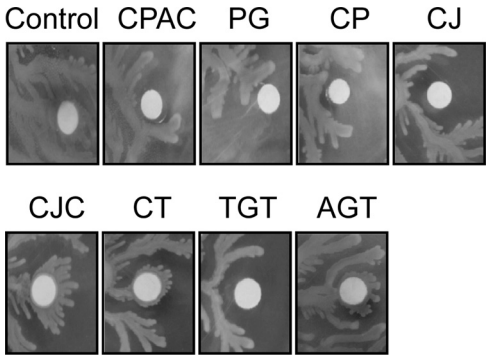


FIG. 5. Representative image of swarming motility on plates containing dried filter paper discs impregnated with various tannin-containing materials. Concentrations of the solutions that the discs were soaked in were as follows: CPAC and PG (0.1 mg/ml), CP (10 mg/ml), and remaining materials (10% [vol/vol]).

were not deterred by CJC, CT, or AGT discs (Fig. 5). This is likely because the effective tannin concentration in the agar surrounding these discs was too low to block swarming motility. The discs themselves did not impair bacterial growth, as when they were placed on a bacterial lawn there was no growth inhibition zone around any of the discs at either 0.5% or 1.5% (wt/vol) of agar (data not shown).

**Supplementing CPAC and PG plates with rhamnolipid (surfactant) partially restored swarming motility.** The results demonstrating that CPAC and PG blocked swarming motility but not swimming or twitching motilities suggested that the compounds are acting on a factor that is required only for swarming motility. One of the key factors required for *P. aeruginosa* swarming motility is the production of a biosurfactant (rhamnolipid) that acts to reduce the surface tension, permitting motility across the surface (5). To assess whether CPAC and PG could be interfering with the levels of surfactant available to the bacterium, we supplemented PAC and PG swarm plates with rhamnolipid (100 μl/ml) and observed whether motility could be rescued. Motility was partially restored in the presence of rhamnolipid (Fig. 6A and B). Furthermore, when discs impregnated in CPAC or PG solutions were placed on plates supplemented with rhamnolipid, the swarming bacteria migrated right up to the discs (Fig. 6C), while they were repelled by the discs on plates without rhamnolipid supplementation

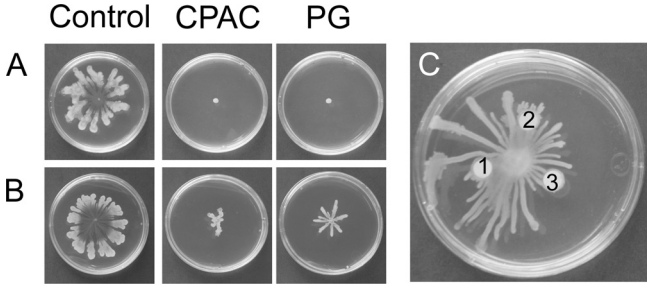


FIG. 6. Representative images of *P. aeruginosa* motility in the presence of CPAC or PG without rhamnolipid supplementation (A) and on plates supplemented with rhamnolipid (B) (100 μl/liter). (C) Swarm motility on plates with rhamnolipid. Discs were impregnated with DI (1), CPAC (2) (100 μg/ml), and PG (3) (100 μg/ml).

TABLE 2. Contact angles of water droplets on swarm agar supplemented with different tannin-containing materials

| Condition or compound | Contact angle (°)[a] | |
|---|---|---|
| | Pre-bacterial inoculation | Post-swarming[b] |
| Swarming motility occurred | | |
| Control | 23.02 ± 2.07 | 0.38 ± 0.08 |
| Turm | 26.80 ± 4.34 | 3.10 ± 0.50 |
| Cinn | 27.46 ± 1.12 | 7.18 ± 3.25 |
| Swarming motility blocked | | |
| CPAC | 35.64 ± 3.23 | 39.68 ± 2.34 |
| PG | 24.60 ± 2.11 | 27.04 ± 3.29 |
| CP | 43.92 ± 3.65 | 62.51 ± 5.90 |

[a] Values shown represent the mean ± SD of 5 contact angles determined with duplicate plates.
[b] Contact angles were determined on the 2-cm agar zone surrounding the active swarming or nonswarming bacteria.

(Fig. 5). This suggests that one of the mechanisms involved in blocking swarming motility is at least rhamnolipid related.

**The agar surrounding nonswarming bacteria was less hydrophilic compared to the agar surrounding active swarmers.** One of the key requirements for agar-associated swarming motility is a wet surface that is largely generated by bacterial rhamnolipid production (5). The hydrophilicity of agar containing different tannin materials was evaluated by measuring the contact angle (CA) of water droplets deposited on the agar surface. The CA is the angle of the droplet edge generated at the agar-air interface, where a low CA implies that the droplet spreads across the agar surface, and therefore, the agar is hydrophilic. In contrast, a high CA correlates with the droplet attempting to distance itself from a surface and hence is hydrophobic.

The CAs of swarm plates without and with rhamnolipid supplementation (100 μl/liter) were determined. Unsupplemented plates resulted in CAs of >20°, whereas rhamnolipid-supplemented plates exhibited CAs of <0.4° (data not shown). This indicated that the presence of rhamnolipid severely increased the "wetness" and hence the hydrophilicity of the agar, as expected. The CAs of agar containing materials that permitted swarming motility (the control, Turm, and Cinn) were then compared to those of plates supplemented with materials that blocked swarming (CPAC, PG, and CP). Before the addition of bacteria, all plates exhibited high CAs of >20°, with CPAC- and CP-supplemented plates exhibiting the highest CAs of >30° (Table 2). Bacteria were then inoculated onto swarm plates, and after incubation (16 h, 37°C), the CA was determined in the 2-cm region of agar surrounding the swarm front or nonswarming central growth. On the control plates (high swarming motility), the CA was very low (<0.5°). For plates supplemented with Turm and Cinn (motility not blocked), the CAs were <10°. In contrast, on plates supplemented with CPAC, PG, and CP (motility blocked), the CAs remained high (>25° for PG, >35° for CPAC, and >60° for CP). This suggests that in the presence of tannins that block swarming motility, the bacteria are not producing a substance (presumably rhamnolipid) that diffuses into the agar to in-

TABLE 3. Summary of the effects of key tannin-containing materials

| Compound | Concn | Impaired swarming | | Impaired growth[c] | |
|---|---|---|---|---|---|
| | | Plates[a] | Discs[b] | Plates | Discs |
| CPAC | 0.1 mg/ml | + | + | X | X |
| PG | 0.1 mg/ml | + | + | X | X |
| CP | 10 mg/ml | + | + | X | X |
| CJ | 10%, vol/vol | NG[d] | + | NG | X |
| CJC | 10%, vol/vol | + | X | X | X |
| CT | 10%, vol/vol | NG | X | NG | X |
| TGT | 10%, vol/vol | + | + | + | X |
| AGT | 10%, vol/vol | + | X | X | X |

[a] + denotes material blocked swarming motility.
[b] + denotes material repelled swarming bacteria. X denotes material did not repel swarming bacteria.
[c] In growth columns, X denotes that growth was not impaired, and + denotes impairment partially occurred, yet colony growth was still evident when the bacterial inoculum was undiluted.
[d] NG, no bacterial growth.

crease the wetness of the agar surface and facilitates swarming motility.

## DISCUSSION

To the best of our knowledge, this is the first study to demonstrate that cranberry PAC, in addition to other tannins, can block bacterial swarming motility and cause migrating bacteria to change direction (data summarized in Table 3). Cranberry products (condensed tannins containing predominantly A-type PACs), pomegranate (hydrolyzable tannin), and green tea extracts (catechins containing B-type PACs) all could block *P. aeruginosa* swarming motility, indicating that this physiological effect is not only specific to A-type PACs. In contrast, experiments conducted with CPAC and PG did not block swimming and twitching motilities, indicating that the tannin compounds are acting upon mechanisms specifically related to swarming motility.

Our data strongly suggested that the majority of these tannin-containing materials blocked swarming motility without impairing *P. aeruginosa* growth capacities. Given that tannin compounds, including PACs, can bind to and precipitate many different types of proteins (14, 15, 29), it is likely that these compounds exert their antiswarming effects via multiple mechanisms. In swimming and twitching motilities, the cells move independently, but swarming requires the bacteria to effectively work together via a process termed "quorum-sensing," involving bacteria sensing the extracellular signals produced by other bacteria (36). Other studies have indicated that tannin compounds can impede bacteria's quorum-sensing system (19, 38). In addition to quorum-sensing, a functional lipopolysaccharide (LPS) is required for *P. aeruginosa* swarming motility (22). Interestingly, both A- and B-type PACs have been found to bind to the LPS of *P. aeruginosa* (7). Hyperflagellation is another requirement for swarming motility (5), and tannin itself is one of the components of the flagellum stain (21). Given the protein binding capacity of tannin compounds, it is feasible that they are also binding to the flagellin subunits, although we did not observe complete blocking of swimming motility by CPAC or PG.

An additional factor required for *P. aeruginosa* swarming motility, and not swimming or twitching motility, is the production of a biosurfactant (rhamnolipid) that acts to reduce the surface tension facilitating motility across the surface (4, 5, 8). In swimming motility assays, bacteria are fully immersed within the semisolid medium, and thus, rhamnolipid is not expected to influence such motility. Our results showed that blocked swarming motility for CPAC and PG could be partially restored by supplementing the plates with rhamnolipid. This suggests that one of the mechanisms contributing to the blocking of swarming is rhamnolipid related. Furthermore, the agar area surrounding actively swarming bacteria exhibited hydrophilic characteristics, implying increased wetness attributable most likely to rhamnolipid production. A moist surface is required for swarming motility to occur (5). In contrast, the agar area of swarming-blocked bacteria was considerably less hydrophilic, indicating the absence of a substance that increases agar moisture content. These findings motivate the pursuit of additional studies to further elucidate the mechanisms by which CPAC and other tannin-containing materials block swarming motility.

Given the importance of swarming motility for conferring biofilm development (25, 10, 35) and antibiotic resistance (3, 4), ongoing research in our laboratory is aimed at investigating the effect of CPAC and other tannin-containing materials on biofilm formation and antibiotic resistance. In addition, it is of interest to explore the potential application of such compounds in limiting bacterial surface colonization in both environmental and clinical settings.

This study focused on *P. aeruginosa* strain PAO1 as a model organism, but this could potentially extend to other organisms that undertake swarming motility, such as *Escherichia coli*, *Vibrio parahaemolyticus*, *Serratia marcescens*, *Salmonella enterica* serovar Typhimurium, and *Proteus mirabilis* (5). This research expands substantially on the work of other studies that have shown tannic acid derivatives can impair the swarming motility of *Burkholderia cepacia* (19) and *Proteus* spp. (30).

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